

NEUROCHEMICAL AND NEUROPHARMACOLOGICAL INVESTIGATION OF
N-CYANOETHYLTRYPTAMINE, A POTENTIAL PRODRUG OF TRYPTAMINE

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Tryptamine (T), a trace amine present in the brain, has been implicated in the etiology of a number of psychiatric disorders (1-3). The existence of T receptors in the mammalian central nervous system has been reported by several workers (2,4-7). Administration of monoamine oxidase inhibiting antidepressants to rodents produces marked increases in brain levels of T (3,8), the relative increases being much greater than observed for the classical amine neurotransmitters.

With a view to studying the functional role of T and 2-phenylethylamine, another trace amine, in the brain we have employed a prodrug approach which utilizes N-alkylated analogs to cause elevations of the respective parent amines (9-11). In the study reported here, N-cyanoethyltryptamine, in which the side chain amino moiety has been alkylated with a 2-cyanoethyl group, has been examined as a prodrug of T.

METHODS: Male Sprague-Dawley rats (170-220 g) were injected with T or CET (0.1 mmol/kg ip) in physiological saline solutions or with physiological saline and groups were sacrificed 5, 15, 30, 60, 90, 120 or 180 min postinjection; brains were removed as quickly as possible and frozen solid in isopentane on solid carbon dioxide. Half of each brain was used for the analysis of T by aqueous pentafluorobenzenesulfonylation followed by gas chromatography with electron capture detection (12). Levels of the neurotransmitter amines noradrenaline (NA), dopamine (DA) and 5-hydroxytryptamine (5-HT) and the acid metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindole-3-acetic acid (5-HIAA) were determined in the other half of the brain samples by high pressure-liquid chromatography with electrochemical detection using a modification of the procedure of Saller and Salama (13). Data were analysed by either one-way or two-way analysis of variance followed by independent t-tests in the case of single pair comparisons and by the Newman-Keuls test in the case of multiple mean comparisons ($\alpha=0.05$). All the probabilities are two-tailed. Pharmacokinetic parameters such as area under the curve (AUC) and half-lives of rapid and slow-elimination phases (α and β respectively) were determined by methods described by Gibaldi and Perrier (14).

RESULTS: Control levels of T in brain samples could not be quantitated accurately by the present method, but they were shown to be less than 1 ng/g. Levels of 0.5 ng/g as reported by a high resolution mass spectrometric procedure (15) were thus assumed to be the control values. There were significant changes in brain T levels following the administration of T and CET [$F(5,30)=45.03$, $p < 0.001$; $F(6,35)=6.91$, $p < 0.001$ respectively] and the multiple mean comparisons are presented in Fig. 1. Levels of T increased significantly above control values following T administration and they were

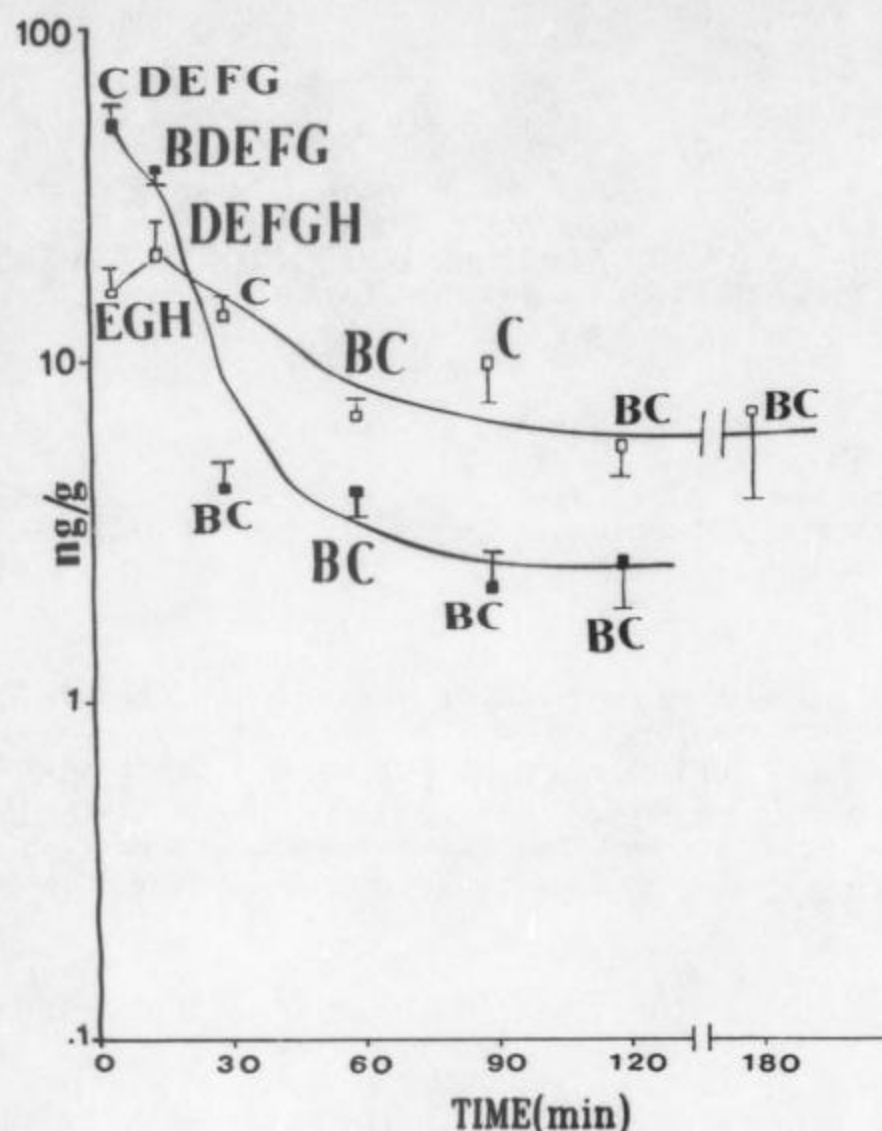


FIGURE 1: Rat brain T levels (ng/g; log scale; mean $\pm$ S.E.M.; n, 5-6) after administration of T (■) and CET (□) (0.1 mmol/kg, ip each). Superscripts A, B, C, D, E, F, G and H refer to significant differences from levels at 0, 5, 15, 30, 60, 90, 120 and 180 min respectively (Newman-Keuls test, $\alpha=0.05$).

approximately 100 times control levels at 5 min. These levels decreased biexponentially with half-lives α and β of 12.5 and 90.0 min respectively, and by 180 min they were undetectable (i.e., <1 ng/g). Following CET administration, concentrations approximately 45 times control levels were obtained at 15 min and they decreased monoexponentially with a β -value of 90 min to approximately 10 times control levels by 180 min. The peak levels of T following CET administration were approximately half of those seen after an equimolar dose of T ($p < 0.005$). The availability of T to brain as measured by AUC (0-120 min) was not significantly different following administration of T and CET (nmol/g min, mean $\pm$ S.E.M., n, 5-6; 7.4 ± 0.62 and 7.8 ± 0.73 respectively, $p > 0.2$), although the patterns of the T increase were much different (see Discussion).

There were significant time dependent changes in NA levels after administration of T [$F(7,42)=2.63$, $p < 0.005$]; at 60 min levels had decreased below control values whereas NA levels at the various time intervals remained unchanged from control values following CET administration. Levels of DA decreased significantly below control values at 5, 30, 60, 90, 120 and 180 min [$F(7,42)=14.08$, $p < 0.001$] after T administration, but increased above control levels at 5, 15 and 30 min [$F(7,44)=5.53$, $p < 0.001$] and decreased to control levels thereafter following CET treatment (Fig. 2). Levels of DOPAC decreased below controls from 5 to 120 min following T injection [$F(7,40)=7.86$, $p < 0.001$], but they increased from 5 to 60 min above control levels [$F(7,44)=$

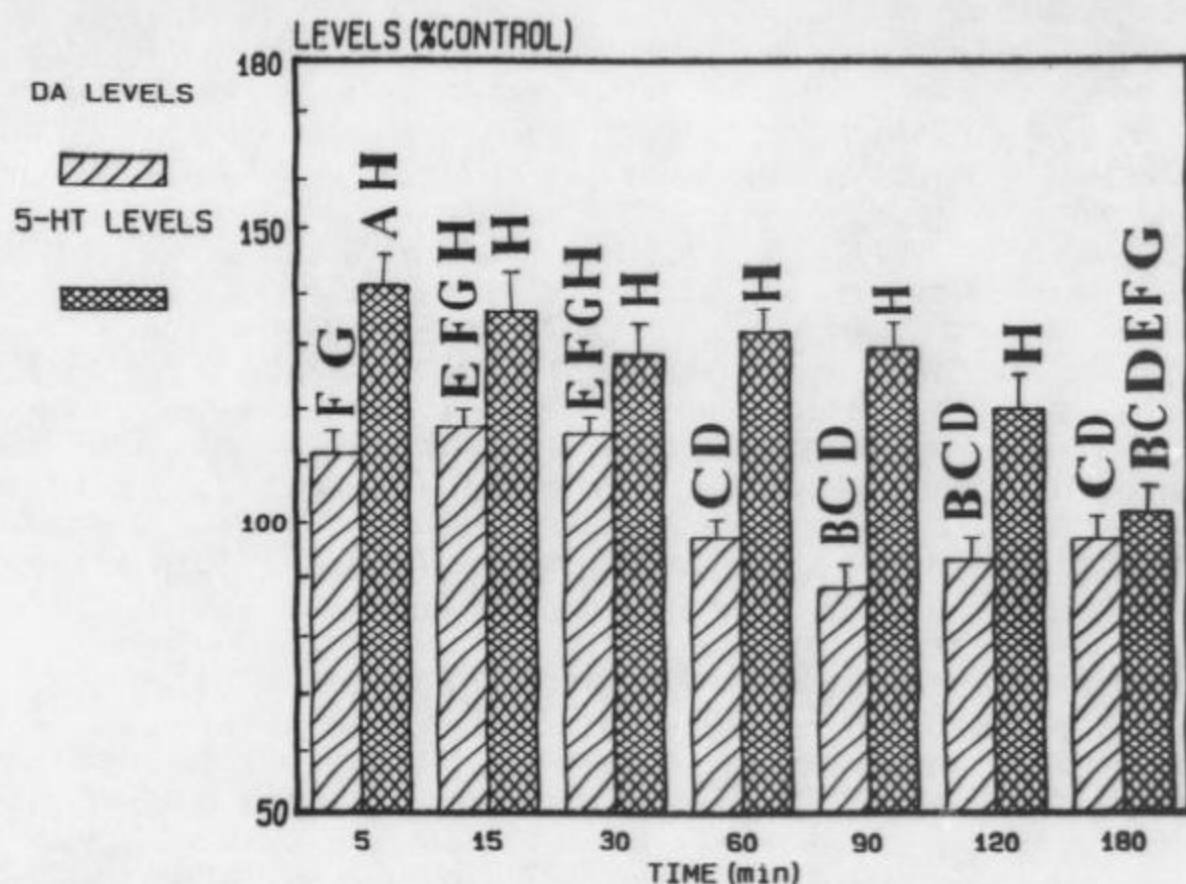


FIGURE 2: Rat brain DA and 5-HT levels (% control, mean $\pm$ S.E.M., n, 5-6) after administration of CET (0.1 mmol/kg, ip). Control DA and 5-HT levels (ng/g, mean $\pm$ S.E.M.) were 680.7 ± 14.7 and 427.3 ± 12.6 respectively. For superscripts refer to Fig. 1.

19.23, $p < 0.001$] after CET treatment. Following T administration, HVA levels increased above control levels at 5 and 15 min and decreased below controls at 120 min [$F(7,42)=10.76$, $p < 0.001$] whereas CET treatment did not affect HVA levels. Administration of T resulted in significant decreases in 5-HT levels below control values at 60 min [$F(7,41)=3.36$, $p < 0.01$], while CET administration caused elevations of 5-HT levels above controls at 5 min [$F(7,43)=6.04$, $p < 0.001$] (Fig. 2). The levels of 5-HIAA remained unchanged following T treatment, but after CET treatment they decreased below control levels at 5, 15, 30 min and increased above control levels at 180 min [$F(7,44)=17.68$, $p < 0.001$].

DISCUSSION: The administration of T itself lead to marked increases in brain T levels, but these decreased rapidly, indicating the unsuitability of using T itself to cause sustained elevations of brain T. The administration of CET resulted in gradual increases in T levels and these were sustained for longer times. Brain T levels could not be detected beyond 120 min following T administration whereas they were nearly 10 times control levels at 180 min after an equimolar dose of CET. Statistical analysis of brain T levels (5-120 min) after T and CET administration indicated a significant difference between effects of drug treatments, a significant effect of time and a significant interaction between drug treatment and time (indicative of differences in concentration time profiles) [$F(1,60)=7.25$, $p < 0.0025$; $F(5,60)=44.76$, $p < 0.001$ and $F(5,60)=16.84$, $p < 0.001$ respectively]. Despite significantly lower peak levels of T, CET treatment provided a similar T availability to brain as did an equimolar dose of T, indicating the delayed release and clearance of T formed from CET. The relative increases in brain T levels after CET were not as high as those seen with 2-phenylethylamine (PEA), another bioactive trace amine, after administration of its N-cyanoethyl analog, CEPEA. This may be due to rapid

elimination of CET in brain in comparison to T formed from it, leading to an apparently weak prodrug effect.

Neurochemical studies indicated fairly potent effects of T and CET on dopaminergic and serotonergic systems with limited effects on the noradrenergic system. Defining turnover of DA as the ratio of (HVA + DOPAC)/DA (% control in all cases; baseline value of 2) and that of 5-HT as 5-HIAA/5-HT (baseline value of 1), the administration of T resulted in increases in DA and 5-HT turnover whereas CET decreased the same. Administration of T resulted in (DOPAC + HVA)/DA values in the range of 2.5-3.3 from 5-90 min; the turnover value decreased to the baseline value of 2 by 180 min. In contrast, CET administration resulted in decreases, with values ranging from 1.06 to 1.5 between 5 and 120 min; by 180 min the value had increased to normal. Administration of T slightly increased the 5-HIAA/5-HT to 1.1-1.34 from 5-180 min, whereas CET administration decreased 5-HT turnover (values in the range of 0.5-0.9) up to 120 min, after which turnover increased to the baseline value. Earlier studies have demonstrated the ability of T to affect the uptake and release of DA and 5-HT (16-20). Increases in DOPAC levels with smaller changes in HVA following T administration suggest a predominance of DA release over inhibition of its reuptake. Decreases in DOPAC levels following CET administration suggest inhibition of reuptake of DA. Preliminary studies indicated CET to be a selective but weak monoamine oxidase (MAO)-A inhibitor *in vitro*, whereas *ex vivo* studies revealed minimal MAO-A inhibition (21), thus suggesting the possibility of reversible inhibition of MAO-A. These observations might explain changes in levels of 5-HT after CET treatment. Besides the possible effect of altered levels of T on neurotransmitter amine uptake and release, the neurochemical effects of CET itself should also be considered. Studies are underway in our laboratories to delineate the effects of CET from those of T formed from it.

In summary, administration of CET resulted in sustained elevations of brain T levels in contrast to the transient changes seen after administration of T itself, suggesting the metabolism of CET to T *in vivo*. Interactions with putative neurotransmitter amines and their metabolites indicated the possibility that CET may be interacting with the transport of these amines at nerve terminals. These metabolic studies with CET should help in designing better prodrugs of T in future.

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REFERENCES

1. Coppen, A., Shaw, D.M., Malleon, A., Eccleston, E. & Grundy, G.: *Br. J. Psychiat.* 111:993 (1965).
2. Dewhurst, W.G.: *Nature (Lond.)* 218:1130 (1968).
3. Dewhurst, W.G.: In *Neurobiology of Trace Amines*. (eds) A.A. Boulton, G.B. Baker, W.G. Dewhurst & M. Sandler, Humana Press, Clifton, 1984, p. 3.
4. Cascio, C.S. & Kellar, K.J.: *Eur. J. Pharmacol.* 95:31 (1983).
5. Wood, P.L., Pilapil, C., La Faille, F., Nair, N.P.V. & Glennon, R.A.: *Arch. Int. Pharmacodyn.* 268:194 (1984).
6. Perry, D.C.: *J. Pharmacol. Exp. Ther.* 236:548 (1986).
7. Altar, C.A., Wasely, A.M. & Martin, L.L.: *Neurosci.* 17:263 (1986).
8. Philips, S.R. & Boulton, A.A.: *J. Neurochem.* 33:159 (1979).
9. Coutts, R.T., Baker, G.B., Nazarali, A.J., Rao, T.S., Micetich, R.G. & Hall, T.W.: In *Neuropsychopharmacology of the Trace Amines*. (eds) A.A. Boulton, L. Maitre, P.R. Bieck & P. Riederer, Humana Press, Clifton, 1985, p. 175.

10. Baker, G.B., Coutts, R.T. & Rao, T.S. (submitted).
11. Rao, T.S., Baker, G.B. & Coutts, R.T.: Prog. Neuro-Psychopharmacol. & Biol. Psychiat. (in press).
12. Rao, T.S., Baker, G.B. & Coutts, R.T.: Biochem. Pharmac. 35:1925 (1986).
13. Saller, C.F. & Salama, A.I.: J. Chromatogr. 309:287 (1984).
14. Gibaldi, M. & Perrier, D.: In Pharmacokinetics, Marcel Dekker, New York, 1982.
15. Philips, S.R. & Boulton, A.A.: Can. J. Biochem. 52:447 (1974).
16. Baker, G.B. & Yasensky, D.L.: Prog. Neuro-Psychopharmacol. 5:557 (1981).
17. Heikkila, R.E. & Cohen, G.: Res. Commun. Chem. Pathol. Pharmacol. 7:539 (1974).
18. Horn, A.S.: J. Neurochem. 21:883 (1973).
19. Baker, G.B., Hiob, L.E., Martin, I.L., Mitchell, P.R. & Dewhurst, W.G.: Proc. West. Pharmacol. Soc. 23:167 (1980).
20. Ennis, A.M. & Cox, B.: Neuropharmacology 21:41 (1982).
21. Rao, T.S.: Ph.D. Thesis, University of Alberta, 1987.